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Journal of Microbiology, Immunology and Infection

Journal homepage: <http://www.e-jmii.com>

Original Article

Comparison of Clinical Features Between Coxsackievirus A2 and Enterovirus 71 During the Enterovirus Outbreak in Taiwan, 2008: A Children's Hospital Experience

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BACKGROUND/PURPOSE: Coxsackievirus A2 (Cox A2) was the predominant serotype in the enterovirus outbreak in Taiwan, 2008. However, detailed clinical features of Cox A2 infection have not been reported. In this study, we compared Cox A2 with enterovirus 71 (EV71) in terms of clinical manifestation and epidemiology during the 2008 enterovirus outbreak in Taiwan.

METHODS: A total of 280 hospitalized patients (97 with culture-proven EV71 infection and 183 with culture-proven Cox A2 infection) in 2008 at the Chang Gung Children's Medical Center were enrolled in this study. Epidemiologic data, clinical manifestations, and outcomes for these patients were collected and compared.

RESULTS: Both Cox A2 and EV71 serotypes peaked in June and declined soon afterwards. Seventy-one percent of the patients were younger than 3 years of age. Both groups had the same male-to-female ratio of 1.6:1. Patients with EV71 infection had a significantly longer hospitalization period (4.1 *vs.* 3.0 days, $p < 0.001$). Fever, fever for more than 3 days with a temperature above 39°C, lethargy, poor activity, poor appetite and a myoclonic jerk were significantly associated with EV71 infection. Fever, or fever with a temperature above 39°C, febrile seizure, elevated white cell counts, and elevated serum C-reactive protein concentrations were significantly associated with Cox A2 infection. Most patients with EV71 infection presented with hand-foot-mouth disease (78.3%), while most Cox A2-infected patients presented with herpangina (83.6%). Central nervous system complications were found in 18.6% of EV71-infected children, but only in 1.1% of Cox A2-infected children. All the patients with Cox A2 infection showed total recovery. One patient with EV71 infection died from encephalitis with cardiopulmonary failure, and 6.2% of EV71-infected children had neurologic sequelae.

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Article History:

Received: Apr 28, 2009

Revised: Jun 1, 2009

Accepted: Jun 15, 2009

CONCLUSION: Both Cox A2 and EV71 serotypes accounted for the enterovirus outbreak in Taiwanese children in 2008. Compared with those infected by EV71, the children with Cox A2 infection mostly presented with herpangina, had fewer central nervous system complications, and had better overall outcome.

KEYWORDS: coxsackievirus A2, encephalitis, enterovirus 71, hand-foot-and-mouth disease, herpangina

Introduction

In the past, enteroviruses were classified into polioviruses, echoviruses, coxsackievirus A and B, and later, the enteroviruses were denoted by numbers, such as enterovirus 68 to 71.¹ In 2000, King et al classified enteroviruses by genomic sequencing as human enteroviruses A to D (4 species).² Since its first isolation in California in 1969, enterovirus 71 (EV71) outbreaks have been reported worldwide.^{3,4} It can cause a wide spectrum of diseases, ranging from febrile illness, hand-foot-and-mouth disease (HFMD), herpangina, meningitis to encephalitis, and even death.⁵⁻⁹

In Taiwan, a large-scale epidemic of EV71 in 1998 caused 78 deaths.⁴ From 2000 to 2001, an epidemic took place and 20–30 deaths were reported. Small-scale outbreaks were seen from 2002 to 2005, and thereafter it seemed to decline for the following 2 years. The epidemic re-surfaced in late 2007 in Southern Taiwan and spread to Northern Taiwan by early 2008.^{10,11} As predicted, a large-scale EV71 outbreak commenced in April 2008.

By June of 2008, an alarming increase in the numbers of coxsackievirus A2 (Cox A2) isolates was reported by our virology laboratory, as well as other contracted virology laboratories of Center for Disease Control and Prevention, Taiwan. Among the enteroviral isolates, EV71 and Cox A2 were the two most common serotypes. Since the detailed clinical features of Cox A2 infection had not been reported before, these findings prompted us to study these clinical features in patients infected with Cox A2. Here, we describe the clinical manifestations, epidemiology, and outcomes of the patients with Cox A2 infection and compare the data with those due to EV71 infection.

Methods

Patients

A total of 714 patients in Chang Gung Children's Hospital, Taoyuan, Taiwan were confirmed (by viral culture) to have

enterovirus infection in 2008. Of these, 193 (27.0%) were Cox A2 infected and 109 (15.3%) were EV71 infected. A total of 183 Cox A2-infected patients and 97 EV71-infected patients were hospitalized and were included in this study. Demographic data such as age, gender, and hospitalization time were collected. Clinical findings including fever, peak body temperatures, symptoms of cough, rhinorrhea, vomiting, diarrhea, myoclonic jerk, febrile and afebrile seizures, limb weakness, poor appetite, poor activity, lethargy, headache, and consciousness change were recorded. Clinical signs including oral ulcers, skin rashes, and laboratory data on admission such as white blood cell (WBC) count, hemoglobin level, platelet count, peak serum C-reactive protein (CRP) concentration, cerebrospinal fluid were routinely collected for comparison. Data regarding the final diagnosis and outcome were also collected.

Diagnostic definitions

Herpangina was defined as the presence of oral ulcers on the anterior tonsillar pillars, and a soft palate, buccal mucosa or uvula. Patients with HFMD had oral ulcers on the tongue or the buccal mucosa, and vesicular rashes over the palms, soles, knees, or buttocks. Fever was defined as body temperature $\geq 38^{\circ}\text{C}$. Leukocytosis was defined as WBC count $\geq 17.5 \times 10^3/\mu\text{L}$. For the complicated cases, meningitis was defined as pleocytosis on cerebrospinal fluid analysis. Encephalitis was characterized by the presence of altered levels of consciousness, personality changes, or hallucinations. Encephalomyelitis included both encephalitis and myelitis-like syndrome, which had the characteristics of acute paralysis, or acute limb weakness with decreased muscle power. Pulmonary hemorrhage was defined as alveolar congestion on a chest X-ray and fresh blood from the endotracheal tube.¹²

Viral isolation and serotyping

Throat and rectal swabs were collected from 714 patients for viral isolation and serotyping using monoclonal antibodies

and neutralization tests. Isolates were cultured with human fetal lung fibroblast (MRC-5), Rhesus monkey kidney epithelial cells (LLC-MK2), human epidermoid cancer cells (Hep-2), and human rhabdomyosarcoma (RD) cells. When enteroviral cytopathic effects affected more than 50% of the cell monolayer, indirect fluorescent antibody staining with panenteroviral antibody (Chemicon International, Temecula, CA, USA) was performed to identify the enterovirus. One-hundred and nine patients were identified as EV71-positive by immunofluorescence using an anti-EV71 monoclonal antibody (Chemicon International). Taiwanese Center for Disease Control and Prevention provided a coxsackie A virus blend for further subtyping of those patients that tested positive for Cox A. Eventually, 193 patients were identified as having Cox A2 infections.¹²

Statistical analysis

Data were analyzed using the SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Student's *t* test was used for continuous variables and the χ^2 test was used for categorical data. A *p* < 0.05 was regarded as statistically significant.

Results

Monthly distribution

Between January and March 2008, no Cox A2-infected case was reported. One case of Cox A2 infection was identified in April. The number of Cox A2-infected cases dramatically increased by June, with 118 cases identified. Afterwards, Cox A2 isolates declined sharply and no further cases were observed by the end of December. As for EV71, cases were found throughout the study period from January to December 2008. Few cases were reported in the early months. Similar to Cox A2, EV71 infection peaked in June, with 28 cases identified. However, unlike Cox A2, EV71 saw a steady rate of detection throughout from July to December (Figure).

Patient demographics

The demographic data of the patients are shown in Table 1. There was no significant difference between the median age of patients with EV71 and Cox A2 infection. The majority of the patients were less than 3 years of age (68.3% in the Cox A2 group and 76.3% in the EV71 group). Gender distribution did not differ significantly between the two groups, both having a male-to-female ratio of 1.6:1. The

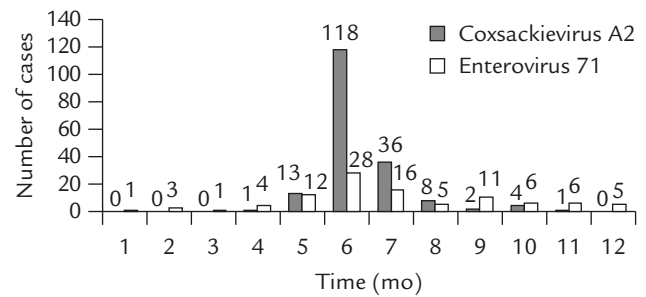


Figure. Monthly distribution of hospitalized patients with coxsackievirus A2 and enterovirus 71 infection.

EV71 group had a significantly longer median hospitalization time than the Cox A2 group (*p* < 0.001).

Clinical manifestation

The clinical manifestations of these patients are summarized in Table 2. Fever for more than 3 days, fever greater than 39°C, fever greater than 39°C combined with fever for more than 3 days, lethargy, poor oral intake, poor activity, a myoclonic jerk, oral ulcers, and skin rashes were significantly more frequent in patients with EV71 infection. In contrast, the presence of fever, fever greater than 39°C, and febrile seizures were significantly more frequent in patients with Cox A2 infection. The incidence and presence of cough, rhinorrhea, vomiting, diarrhea, and headache were similar between the two groups. Ataxia, limb weakness and an unsteady gait were only seen in those with EV71 infection.

Laboratory data

Compared with those with EV71 infection, patients with Cox A2 infection had a significantly higher median WBC count and median CRP level. Moreover, leukocytosis and CRP > 40 mg/L were significantly more frequent in patients with Cox A2 infection (Table 2). In terms of hemoglobin level and platelet count, there were no significant differences between the two groups.

Final diagnosis

Table 3 illustrates the final diagnoses of these patients. A total of 78.4% of the patients with EV71 infection manifested as HFMD, while 83.6% of the patients with Cox A2 infection had herpangina. Of the patients with EV71 infection, 18 (18.6%) had central nervous system (CNS) involvement, while only two (1.1%) patients with Cox A2 infection had CNS involvement.

Table 1. Comparison of the demographics between the patients with coxsackievirus A2 and enterovirus 71^a

Variables	EV71 (<i>n</i> =97)	Cox A2 (<i>n</i> =183)	<i>p</i>
Age (yr)	1.9 (0.54–11.54)	2.1 (0.08–14.83)	0.285
Age <3 yr	74 (76.3)	125 (68.3)	0.250
Sex, M:F	1.6:1	1.6:1	0.903
Length of hospitalization (d)	4.1 (1–63)	3.0 (1–16)	<0.001

^aData presented as median (range) or *n* (%). M=Male; F=female.

Table 2. Comparison of clinical manifestations and laboratory data between the patients with coxsackievirus A2 and enterovirus 71^a

Symptoms	EV71 (<i>n</i> =97)	Cox A2 (<i>n</i> =183)	<i>p</i>
Fever	92 (94.8)	182 (99.5)	0.005
≥3 days	62 (63.9)	60 (32.8)	<0.001
≥39°C	51 (52.5)	134 (73.2)	<0.001
≥3 days & 39°C	39 (40.2)	45 (24.6)	0.017
Cough	21 (21.6)	63 (34.4)	0.031
Rhinorrhea	21 (21.6)	51 (27.9)	0.273
Vomiting	28 (28.8)	69 (37.7)	0.120
Diarrhea	6 (6.1)	18 (9.8)	0.358
Lethargy	23 (23.7)	16 (8.7)	0.001
Poor oral intake	77 (79.3)	119 (65.0)	0.025
Poor activity	59 (60.8)	85 (46.4)	0.037
Myoclonic jerk	48 (49.4)	53 (28.4)	0.001
Febrile seizure	2 (2.0)	16 (8.7)	0.038
Altered consciousness	8 (8.2)	4 (2.2)	0.047
Ataxia	5 (5.1)	0	0.037
Limb weakness	6 (6.1)	0	0.004
Unsteady gait	2 (2.0)	0	0.335
Oral ulcer	93 (95.8)	159 (86.9)	0.023
Skin rash	83 (85.5)	17 (9.3)	<0.001
WBC count (/μL)	12,200 (5.1–24.6)	14,200 (2.9–33.3)	0.001
WBC=17,500/μL	11 (11.3)	46 (25.1)	0.006
CRP (mg/L)	9.43 (0.4–103.4)	30.00 (0.5–243.0)	<0.001
CRP >40 mg/L	9 (9.3)	77 (42.1)	<0.001

^aData presented as *n* (%) or median (range). WBC=White blood cell; CRP=C-reactive protein.

Outcome

Ninety patients (92.8%) with EV71 infection showed complete recovery. Six patients (6.5%) had sequelae, including limb weakness in two patients, epilepsy in three patients, and two-limb disability (one upper limb and one lower limb) due to the complication of post-extracorporeal membrane oxygenation, with resultant amputation in one patient. The

only one fatal case was a 3 year-old male who was admitted for fever, vomiting, lethargy and a frequent myoclonic jerk. He developed myocarditis and pulmonary hemorrhage soon after admission and deteriorated rapidly. Despite the use of post-extracorporeal membrane oxygenation, he eventually died after 56 days of hospitalization. All patients with Cox A2 infection had complete recovery without sequelae (Table 3).

Table 3. Comparison of final diagnosis and outcome between the patients with coxsackievirus A2 and enterovirus 71^a

Diagnosis	EV71 (<i>n</i> =97)	Cox A2 (<i>n</i> =183)	<i>p</i>
Uncomplicated			
Herpangina	16 (16.5)	153 (83.6)	<0.001
HFMD	76 (78.4)	8 (4.4)	<0.001
Pharyngotonsillitis	1 (1.0)	13 (7.1)	0.040
URI	2 (2.1)	4 (2.2)	1.000
AGE	0	1 (0.5)	0.737
Bronchopneumonia	0	2 (1.1)	0.542
Seizure	2 (2.1)	1 (0.5)	0.260
Complicated ^b	18 (18.6)	2 (1.1)	
Aseptic meningitis	2 (2.1)	0	0.111
Encephalitis	6 (6.2)	1 (0.5)	0.017
Encephalomyelitis	7 (7.2)	1 (0.5)	0.006
Pulmonary hemorrhage	1 (1.0)	0	0.335
Myocarditis	1 (1.0)	0	0.335
Acute paralysis	1 (1.0)	0	0.335
Outcome			
Recovery	90 (92.8)	183 (100)	0.005
Sequelae	6 (6.2)	0	0.018
Death	1 (1.0)	0	0.263

^aData presented as *n* (%); ^b≥2 diagnoses. HFMD = Hand-foot-and-mouth disease; URI = upper respiratory infection; AGE = acute gastroenteritis.

Discussion

In this study, we found that the clinical features are different between these two viral serotypes. Patients with Cox A2 infection mostly presented with herpangina, and rarely had CNS complications. In contrast, patients with EV71 infection mostly presented with HFMD, had more CNS complications (even resulting in cardiopulmonary failure with fatal outcome), and had more neurologic sequelae.

More than 95% of the patients in this study, either with Cox A2 or EV71 infection, had fever. Two-thirds of the patients with EV71 infection even had fever for more than 3 days, which is consistent with our previous findings.¹² Lethargy and myoclonic jerk, both indicative of CNS involvement, were more frequently seen in patients with EV71 infection. Forty-nine percent of patients with EV71 infection in this study had a myoclonic jerk, which is compatible with our previous observations.¹³ However, it is intriguing that febrile seizures were significantly more frequent in patients with Cox A2 infection at a rate of 8.7%, which is higher than that in the general population

of this age (~5%). Since lumbar puncture was not performed in most of these cases, we cannot link CNS infection directly to these patients. Other nonspecific symptoms such as cough, rhinorrhea, vomiting, and diarrhea were not significantly different between the two groups.

In this study, more than three quarters of EV71-infected patients manifested as HFMD; most patients with CNS complications also initially presented as HFMD. These findings are consistent with previous observations from Taiwan.^{12–16} In contrast, herpangina was the most common diagnosis for Cox A2-infected patients.

About 20% of hospitalized EV71-infected patients had CNS involvement in this study, but only one patient (1%) progressed to cardiopulmonary failure and died. In our previous studies,^{12–15} the rate of CNS complications among EV71-infected hospitalized patients ranged from 21% to 32%, and the rate of progression to cardiopulmonary failure ranged from 8% to 11%. This discrepancy in CNS complication rate may be explained by several possibilities. First, the indication and the bed availability for hospitalization may be different at different times, with the result that

complication rates were different. Second, the genotype of the EV71 isolates circulating in Taiwan changed within the past decade; genotype C2 and B4 dominated the 1998 epidemic, then B4 prevailed in 1999–2003, C4 emerged in 2004–2005, C5 in 2006–2007, and B5 in 2007–2008.^{17,18} Further studies are required to ascertain whether the virulence of the different genotypes is different. Third, the clinical practice may have been different at different times; the indication and principle for the performance of a lumbar puncture, for example. A lower lumbar puncture performance rate may result in a lower diagnosis rate of aseptic meningitis. In contrast, only 1.1% of Cox A2-infected patients had CNS complications, and none progressed to cardiopulmonary failure.

All the patients in this study with Cox A2 infection, including the two patients with encephalitis and encephalomyelitis, recovered without any sequelae. Total recovery was achieved in 93% of the EV71-infected patients, and neurologic sequelae were identified in 6.2% of EV71-infected patients. In our previous study, the rate of neurologic sequelae among EV71-infected hospitalized patients between 2000 and 2002 was as high as 82%.¹⁹ The better outcomes seen in 2008 might be due to fewer patients progressing to cardiopulmonary failure, as well as a well-practiced staged-based management program.^{19,20}

However, there are several limitations to this study as it is a retrospective study in nature. First, virus isolation and identification was not performed in every patient with possible EV71 infection who visited our hospital during the study period. Second, not every patient with Cox A2 or EV71 infection was hospitalized and, subsequently, were not included in the study. Therefore, the clinical features shown here cannot represent the whole picture for children with Cox A2 infections, but only those that were hospitalized.

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